Fig. 1

Fig. 1. Peroperative identification of sentinel node(s). Identification of sentinel nodes was performed by subserosal injections of Patent blue dye (A) at four sites around the tumor (B). Usually within five minutes, one or more blue coloured lymph nodes appear in the mesentery (C).

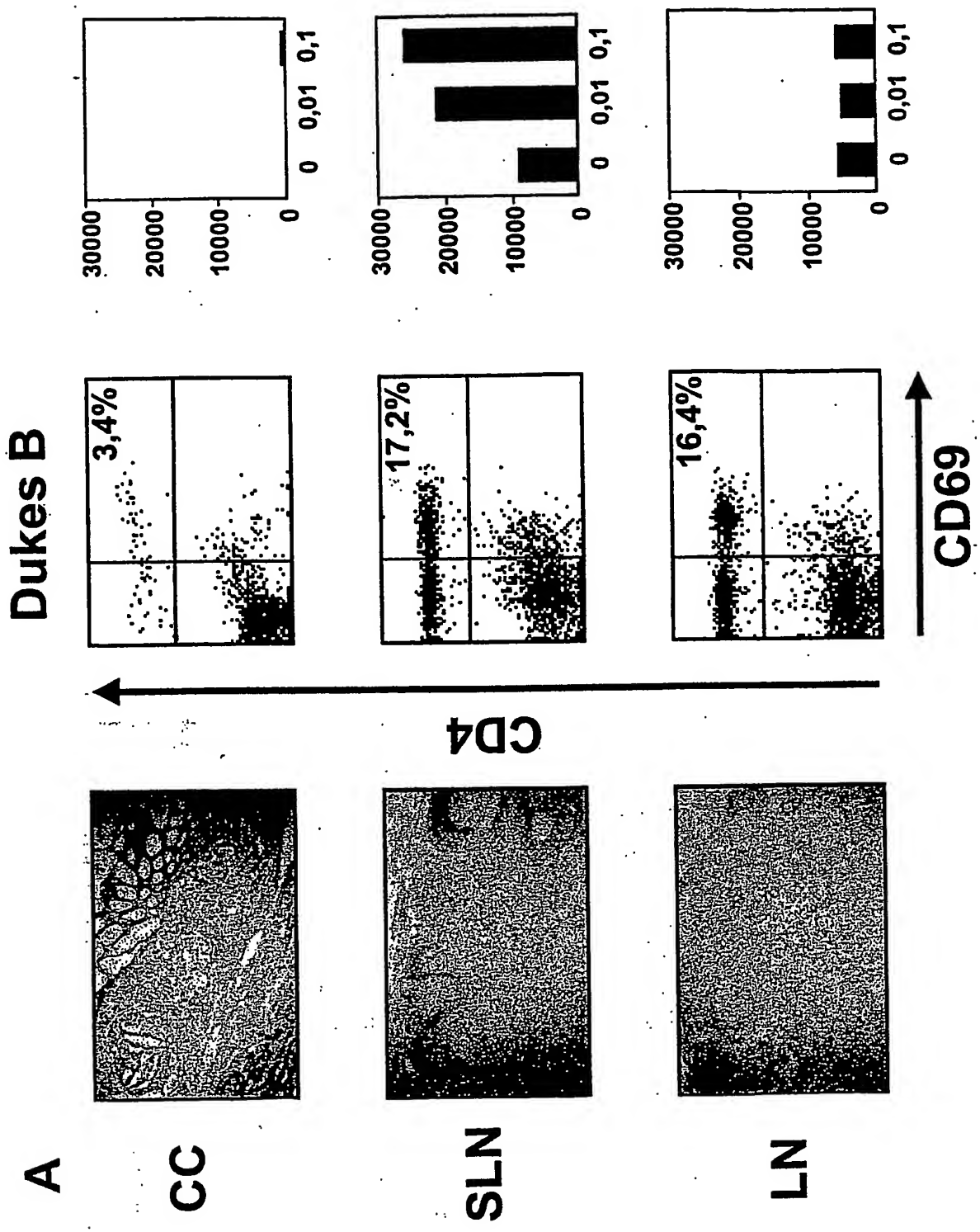
A
CC
SLN
LN
CD4
CD69
Dukes B
3,4%
17,2%
16,4%
30000
20000
10000
0
0
0,01
0,1

Fig. 2A

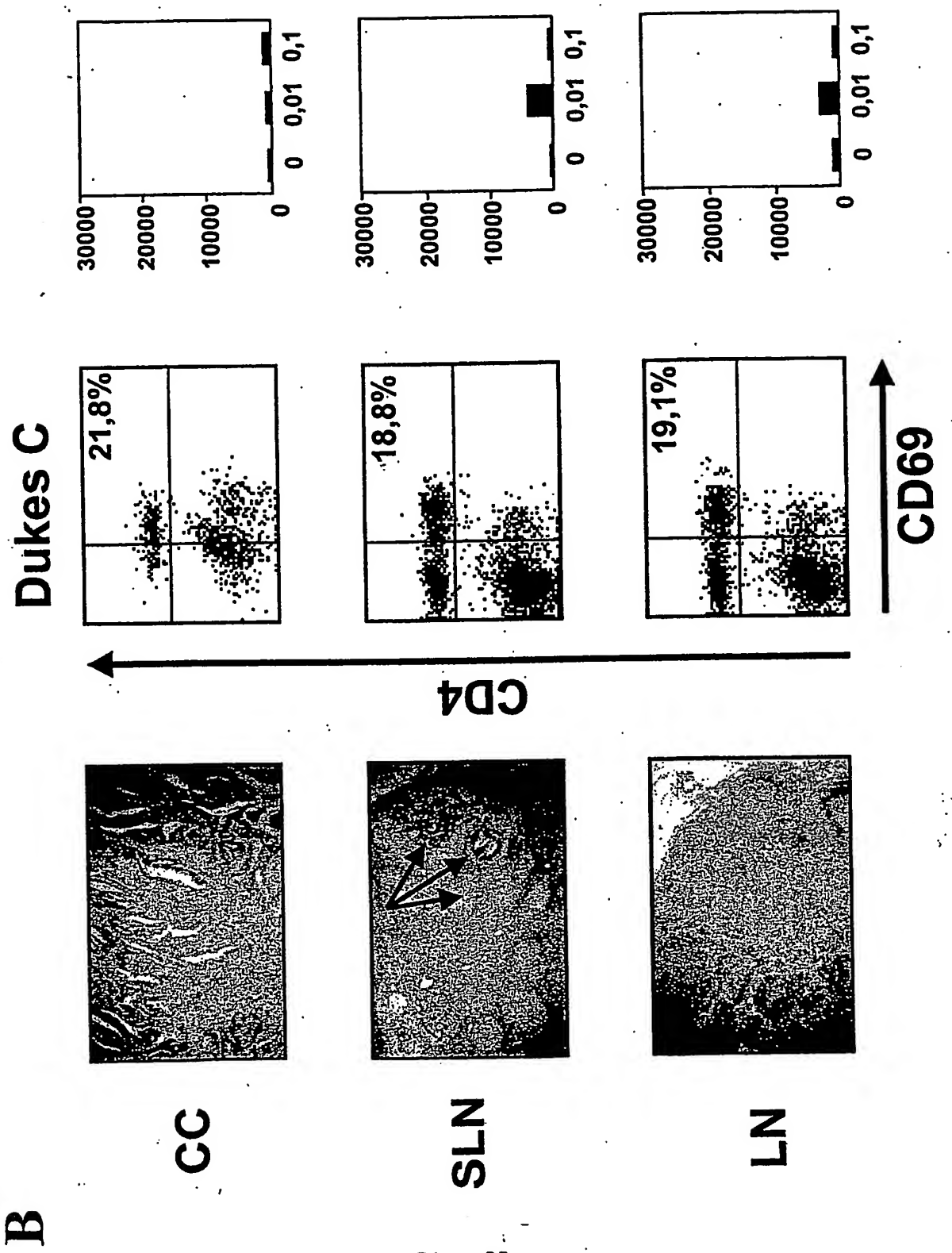
B
CC
SLN
LN
CD4
CD69
Dukes C
21,8%
18,8%
19,1%
30000
20000
10000
0
0
0,01
0,1

Fig. 2B

Fig. 2A + 2B. Characterization of lymphocytes. Data from a patient with Dukes B (A) and Dukes C (B). Specimens from the tumor (CC), sentinel node (SN) and non-sentinel node (LN) were stained with hematoxylin-eosin (left panels) (40x). Arrows indicate the presence of metastatic colon cancer cells in a sentinel node (B, left panel, SN). Lymphocytes from the tumor (CC), sentinel node (SN), non-draining lymph nodes (LN) were stained with antibodies against CD4 and the activation marker CD69 and analyzed using flow cytometry (middle panels), the percentage of double positive activated CD4 T helper cells are indicated in the upper right corner. Cells from the tumor, (CC), sentinel node (SN) and lymph node (LN) were incubated with a 10- or 100-fold dilution of autologous colon cancer extract in a day 5-7 time course study. Cells were pulsed 16h before harvesting with 1 μCi 3H-Thymidine. Peak proliferation occurred at day 5 (right panels).

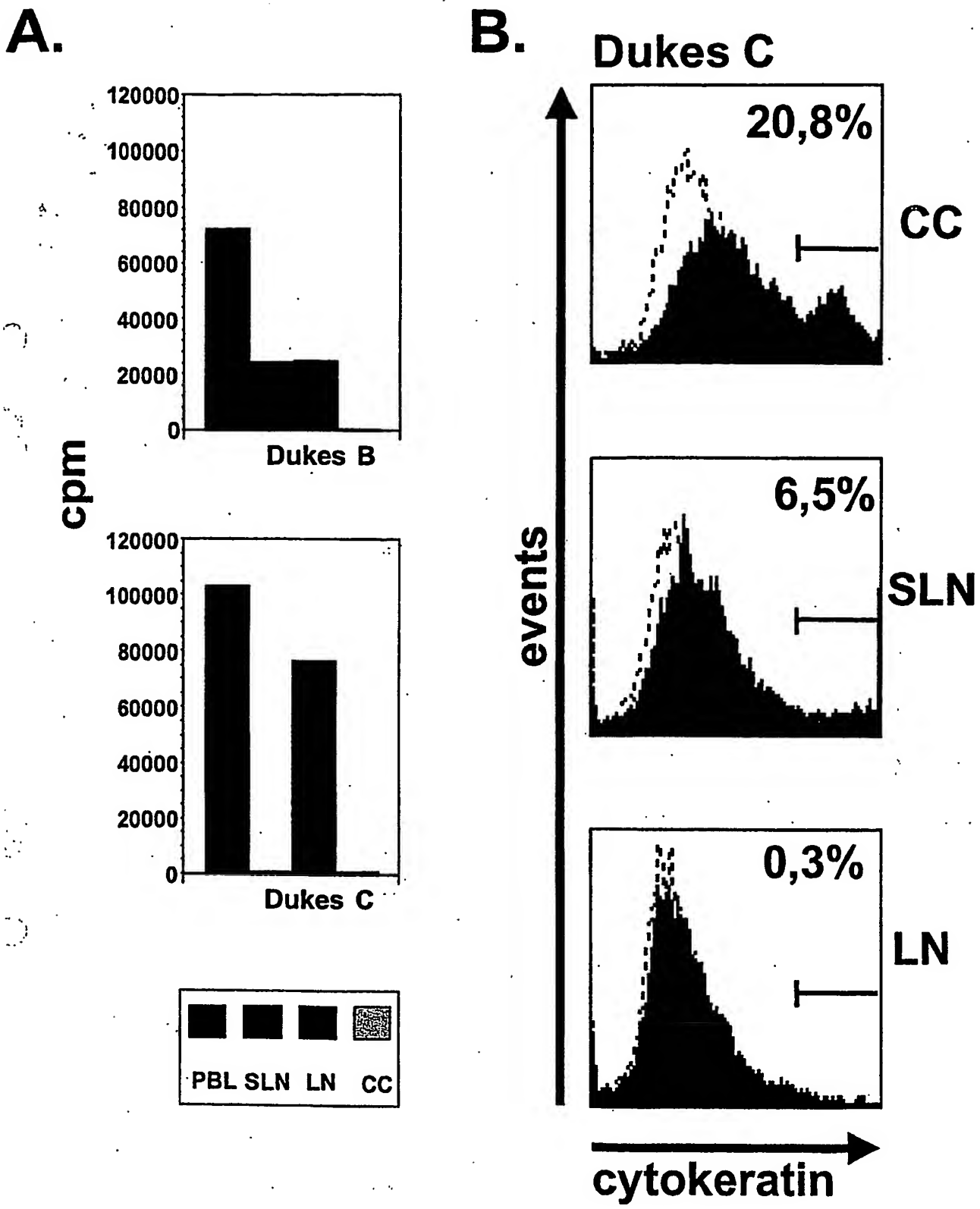
A.
120000
100000
80000
60000
40000
20000
0
cpm
Dukes B
Dukes C
PBL SLN LN CC
B.
Dukes C
20,8%
CC
6,5%
SLN
0,3%
LN
events
cytokeratin

Fig. 3

Fig. 3. Unspecific activation and intracellular FACS analyses for metastasis. Proliferative responses against Concanavalin A stimulation (A) and intracellular FACS analyses using cytokeratin-20 antibody (B). Cells from peripheral blood (PBL), the tumor (CC), sentinel node (SN) and lymph node (LN) were investigated in a time course proliferation assay stimulating with 10 μg/mL of Concanavalin A (A). Cells from the tumor (CC), sentinel node (SN) and lymph node (LN) were permeabilized with saponin followed by incubation with an anti cytokeratin-20 antibody and detection with an anti mouse IgG FITC conjugated antibody (B). The dotted line represent control samples incubated with secondary antibody only and in the overlay plot both the primary and secondary antibodies were included in the incubations. The numbers indicate the percentage of cytokeratin-20 positive tumor cells.

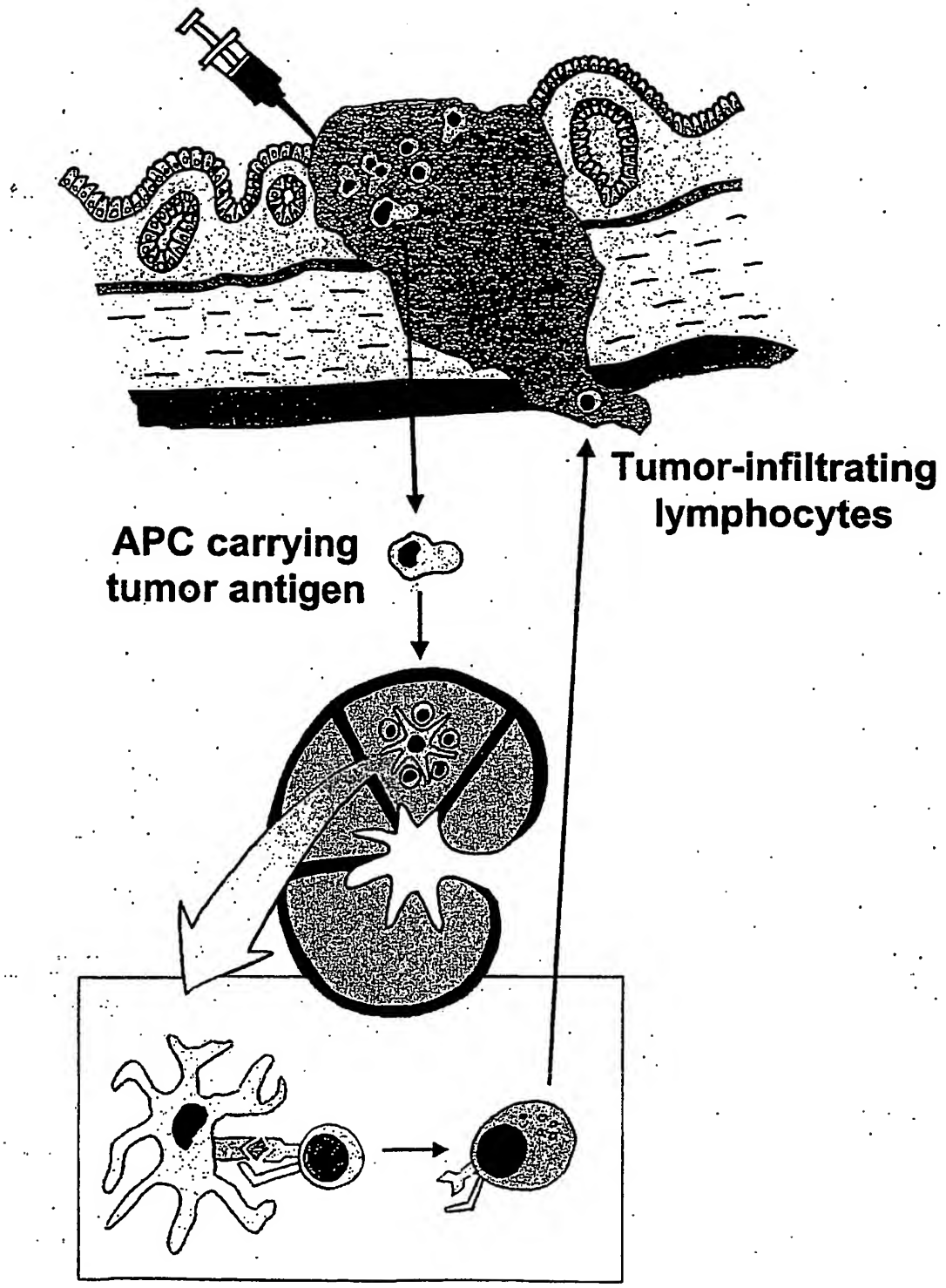
Tumor-infiltrating lymphocytes
APC carrying tumor antigen
Antigen-presentation and subsequent activation of T-lymphocytes

Fig. 4A

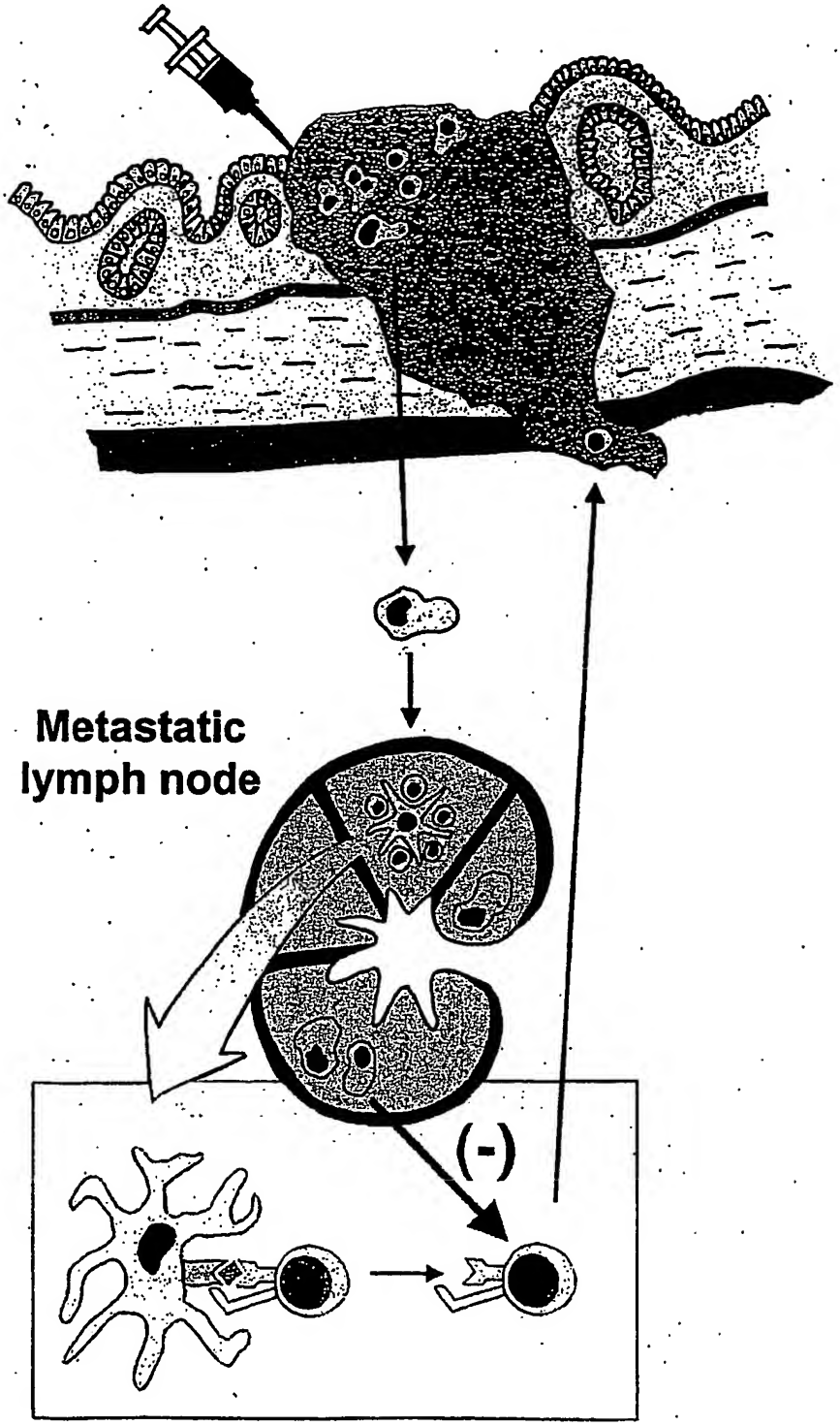
Metastatic
lymph node
(-)
Presence of lymph node metastasis
supress activation of T-lymphocytes

Fig. 4B

Fig. 4A + 4B. Hypothetical scheme Dukes B (A). In the tumor there is a rapid turnover of cells, lack of oxygen and nutritions causing a hostile environment attracting macrophages and dendritic cells. Debris from tumor cells are phagocytosed by these professional antigen presenting cells (APC) and transported by the lymph vessels to the draining sentinel node, which can be detected peroperatively by peritumoral injection of Patent blue. In the sentinel node the APCs load peptides, processed in the endosomal/lysosomal pathway and derived from tumor antigens, mainly into the HLA class II pocket. The class II-tumor peptide complex is transported to the surface of the APC and is recognized by CD4+ T helper cells which provide the first activation signal. The second activation signal is provided by costimulatory molecules such as B7.1, B7.2 and ICAM-1 that are expressed at high levels on the APC. Thus the CD4+ T helper cell becomes an activated effector cell and leave the lymph node to return to the blood stream via the thoracic duct. The activated CD4+ T helper cell leave the blood stream in areas of inflammation and new vessel formation such as in a tumor or metastases. The cell now becomes a tumor infiltrating lymphocyte (TIL). However, due to the local hostile environment in the tumor and possibly by immunosuppressive cytokines released from the tumor the TIL cells frequently become immunosuppressed and unresponsive (anergic).

Dukes C (B): In the presence of metastatic cells in a sentinel node we find that lymphocytes are unable to proliferate against tumor extract nor against an unspecific activator such as Concanavalin A. APCs with phagocytosed tumor debris are likely to be present and the cells seem to be activated. One explanation is that metastatic cells produce immunosuppressive factors. Another explanation is that Dukes C (presence of lymph node metastases) only occurs in patients with an immune failure to recognize the tumor as foreign. Thus a similar situation as seen in different mice strains challenged with Leishmaniasis where some strains clear the infection whereas others succumb due to genetical background differences.